Aging & Rehabilitation

An Interdisciplinary Research Seminar Series

Sponsors

Department of Veteran Affairs

- Rehabilitation Outcomes Research Center (RORC)
- Brain Rehabilitation Outcomes Research Center (BRRC)
- Geriatric Research, Education, and Clinical Center (GRECC)

UF College of Medicine

- Institute on Aging
- Department of Aging and Geriatric Research

UF College of Public Health and Health Professions

- **Brooks Center for Rehabilitation Studies**

UF College of Liberal Arts and Sciences

- Center for Gerontological Studies

UF McKnight Brain Institute

UF College of Nursing

Schedule

- January 9th, 2006 – May 22nd, 2006
- Mondays, 12:00 – 1:00
- Location: UF HPNP Building, Room G101
- Cyber Seminar:
  - VA RORC Conference Room, Commerce Building Downtown
  - VA BRRC Nursing Home Care Unit Conference Room (first floor)
  - UF Brooks Center Conference Room, Jacksonville (904) 306-8977

Themes

- Basic Science
- Clinical Science
- Outcomes / Health Policy
- Behavioral and Social Research
- Cutting Edge / New Research

Iron-containing deposits and neurodegeneration

Chris Batich, PhD
Materials Science and Engineering Dept.
392-6630
March 20, 2006

Iron in the CNS, a summary

- Iron occurs in two principal oxidation states: **ferrous (Fe^{2+}) and ferric (Fe^{3+})**
- Iron stored in **inactive form** bound to protein: 90% of non-heme Fe content of brain is associated with **ferritin**
- The ferritin core primarily is a hydrated iron oxide **($5Fe_2O_3 \cdot 9H_2O$)** ferrihydrite containing **ferric form (Fe^{3+})**
- **Biogenic magnetite (Fe_3O_4) discovered in human brain tissue**

- **Ferritin cores from AD tissue contain structures similar to iron oxides such as:**
  - hematite (α-Fe_2O_3)
  - maghemite (γ-Fe_2O_3)
  - magnetite (Fe_3O_4)

"Magneto-ferritin" cores show two coexisting iron forms: **Fe^{2+} and Fe^{3+}**

Forms of iron in tissue bound to protein (4g)

Functional:		**mol. wt. (kD)**
60 %	hemoglobin (Hb)	16
8 %	myoglobin	16.9
5 %	enzymes	varies

Storage:

20 %	ferritin	470
5%	hemosiderin	[degraded Hb/ferritin?]
0.2%	transferrin (transport)	79.6

Other protein-containing aggregates have high levels of iron

Pathological iron-containing proteins:

lipofuscin [degraded mitochondria?]

polyQ (e.g., Huntington's disease)

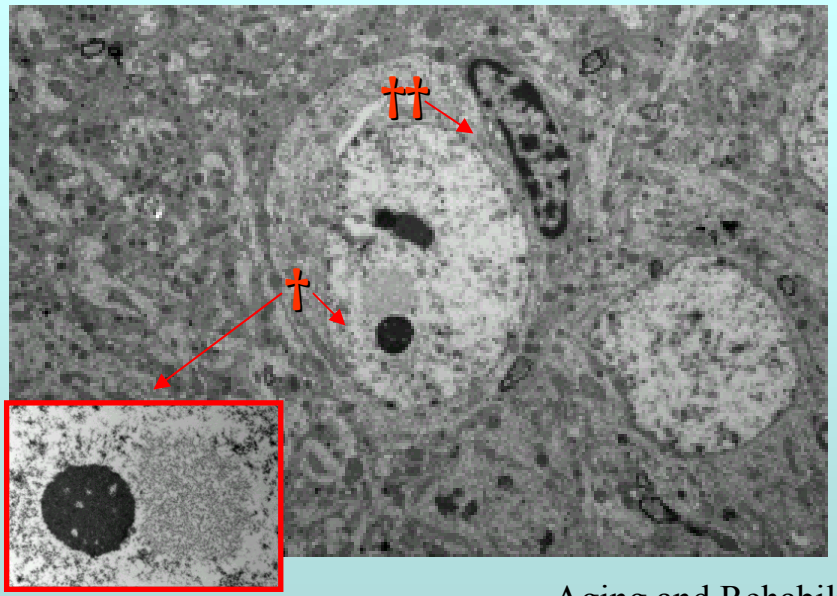
synuclein (PD)

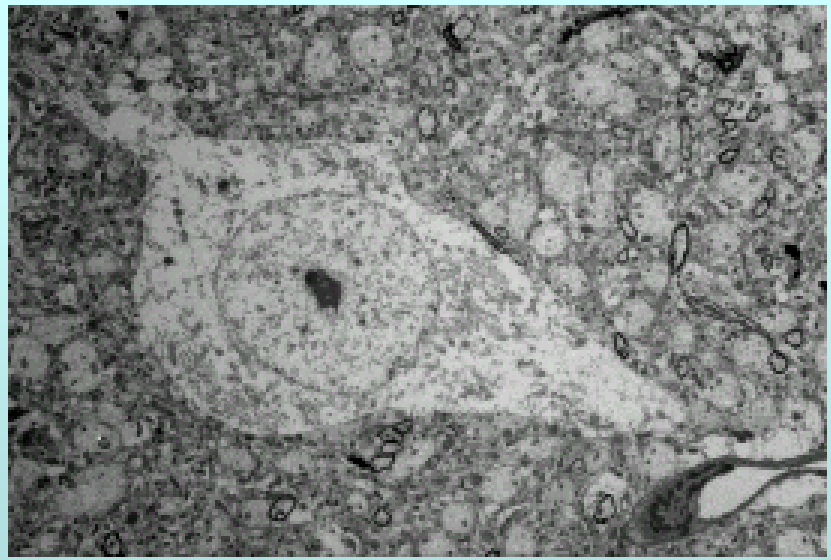
amyloid and tau (AD)

Neuropathological Changes in Brain of R6/2 Mouse

- Transmission Electron Micrograph of neuron with NII † and peri-neuronal glial cell ††. Magnification X2000
- Filamentous neuronal intranuclear inclusion. Magnification X20000

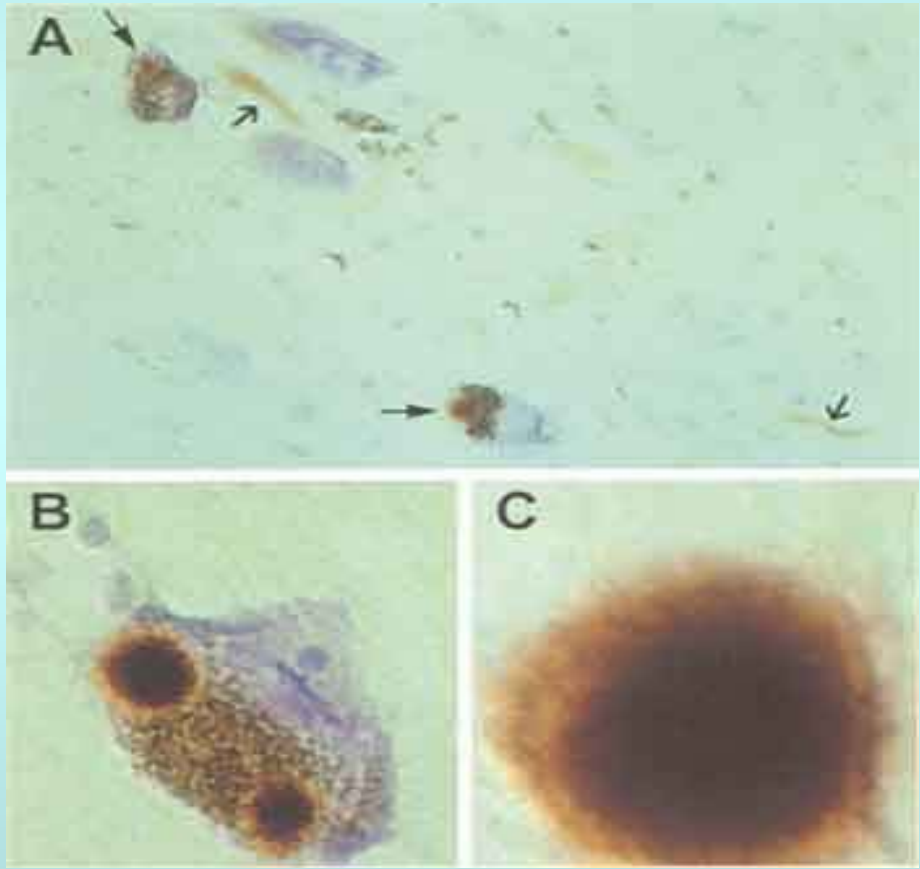
- Transmission Electron Micrograph of neuron found in control mouse tissue.



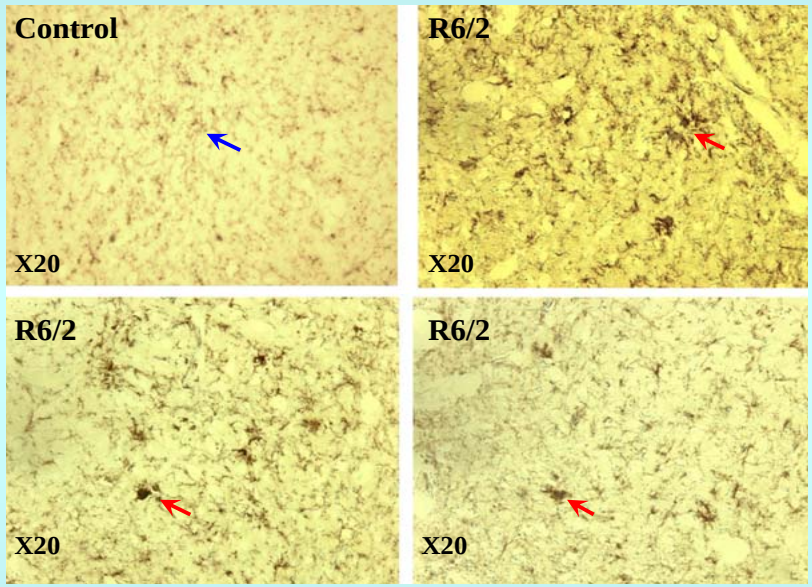


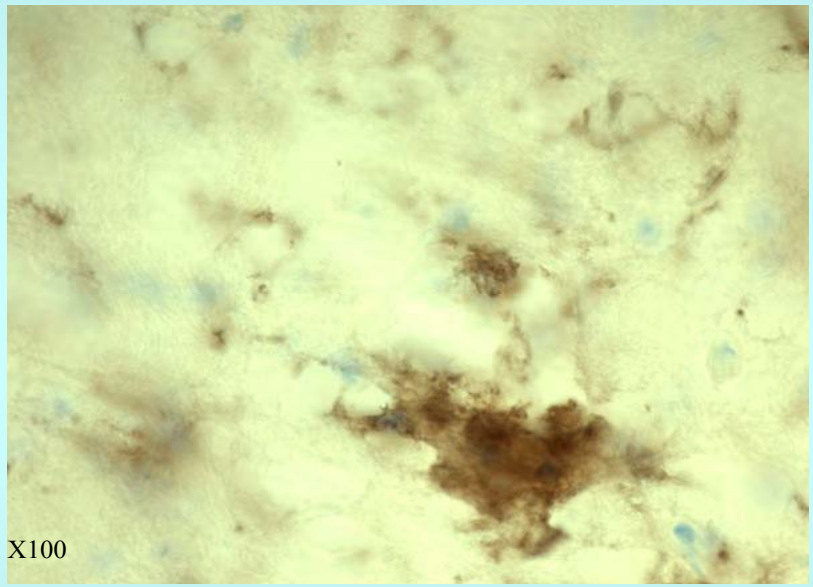
Small, dense deposits, termed Lewy bodies or Lewy neurites, stud the brains of patients with Parkinson's disease. The three sections of brain tissue shown below depict how the protein, alpha-synuclein (stained brown), packs these deposits.

http://web.sfn.org/content/Publications/BrainBriefings/parkinson.html#fullsize



Neuropathological Changes in Brain of R6/2 Mouse

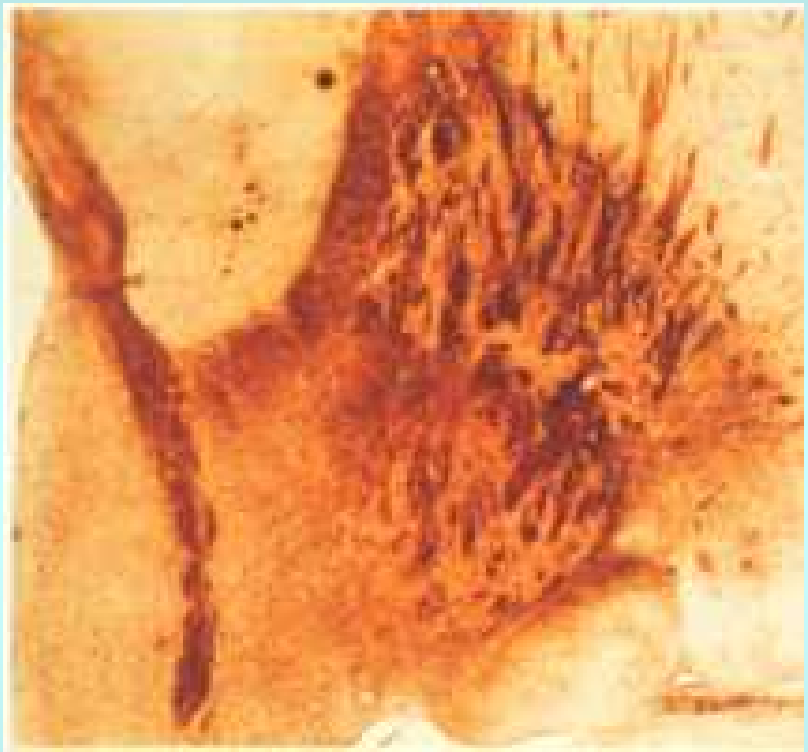




Microglia appearance in control and R6/2 brain tissue

Compare to resting microglia in control tissue, microglia in R6/2 tissue show enhanced staining and bushy appearance. Localized clustering may be an indication of ongoing pathology

Iron deposits in aging brain tissue



***Dhenain**, et al.*
Neurobiol Aging. 1998 Jan-Feb;19(1):65-9.

On T2-weighted magnetic resonance images, the signal intensity is decreased in the pallidum and substantia nigra of aging mouse lemurs in comparison to young animals.

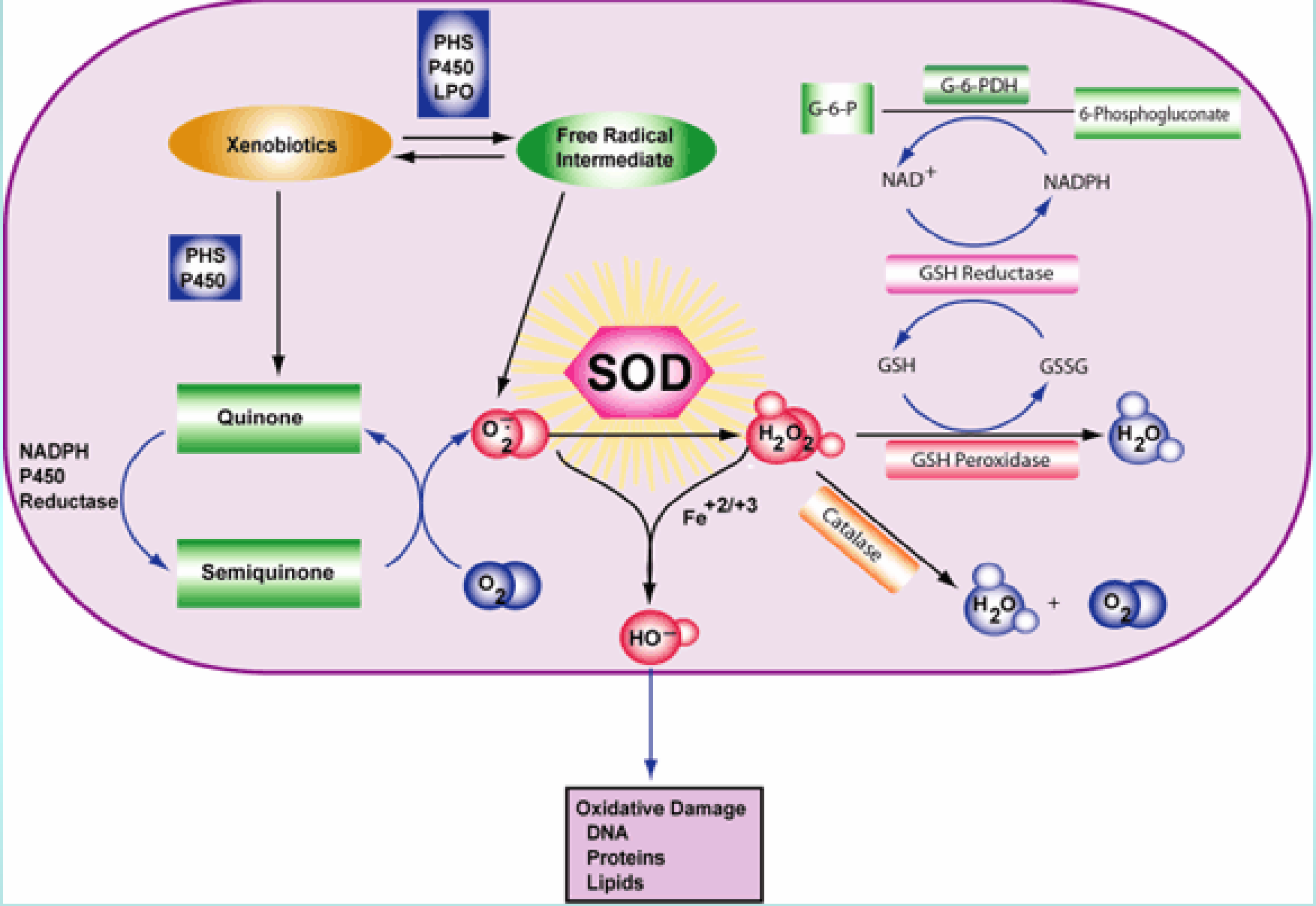
Iron can promote damaging oxidation in several ways:

- **"Fenton" chemistry (oxidative catalysis)**

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + HO\cdot + HO^-$$

Homogeneous (solution)
Heterogeneous (on solid particles)

- **As well as possible magnetic field effects**



http://www.sigmaaldrich.com/Area_of_Interest/Biochemicals/Enzyme_Explorer/Cell_Signaling_Enzymes/Superoxide_Dismutase.html

Removal of iron may improve cognitive function

Iron deposits in children with cerebral malaria were treated with an iron chelator, when in a coma. Measured hours to become conscious

untreated: 43
treated: 20

Gordeuk et al., *Effect of iron chelation therapy on recovery from deep coma in children with cerebral malaria.*
N Engl J Med. 1992 Nov 19;327(21):1473-7

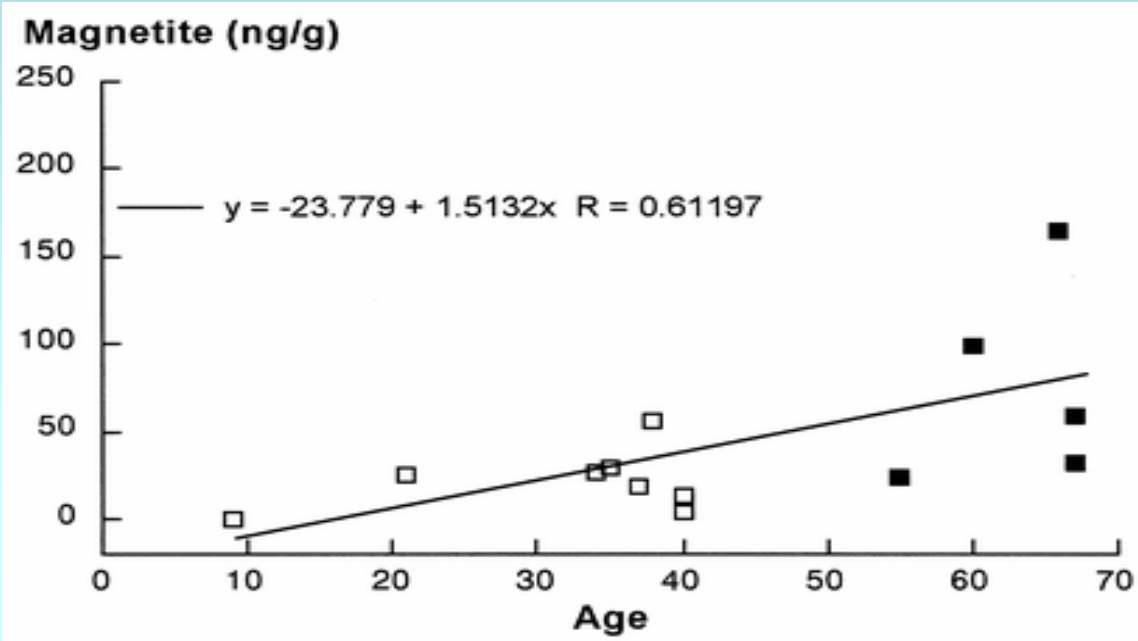
Current state of the knowledge

"The physiological iron complex is unknown, consequently the rate constants for the …oxidation by hydrogen peroxide are unknown."

W. Koppenol

The Oxygen Society Annual Meeting, San Diego (2000) pg. 19

Magnetite increases in the brain with age



Tissue concentration of magnetite (nanograms of magnetite per gram of tissue) versus subject age for all males. *Solid squares* are non-epileptic subjects, *open squares* are epileptic subjects (*n*=14). b Tissue concentration of magnetite for all male subjects minus subject KK (*n*=13)

J. Dobson, Exp Brain Res. 2002 May;144(1):122-6

"Needle in a Haystack" problem

Question: How to identify minute deposits in large samples?

Answer: Very sensitive synchrotron methods (XAFS, XANES), followed by Transmission Electron microscopy (TEM)

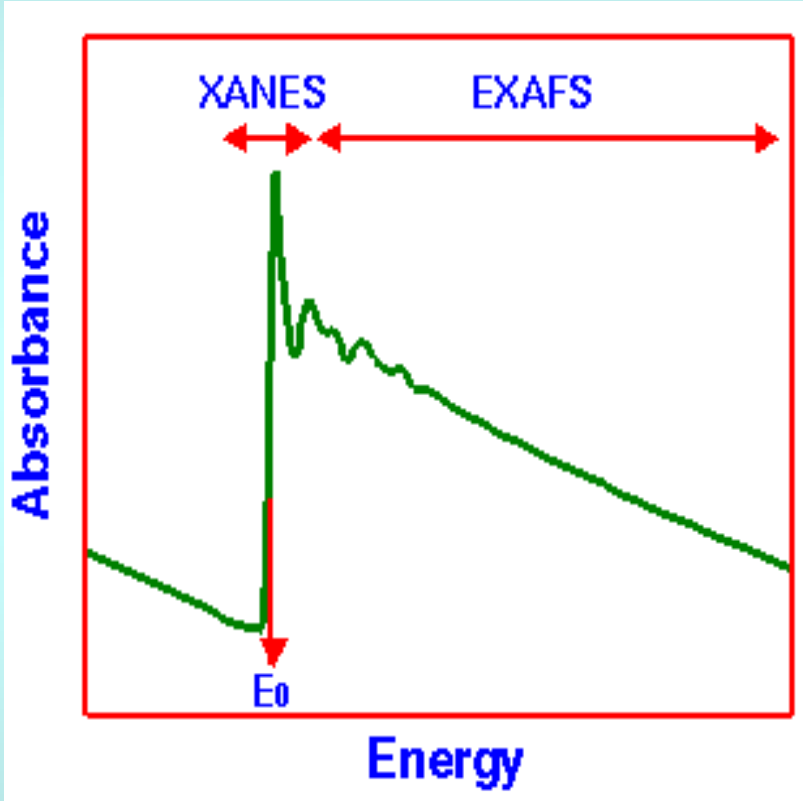
XAFS: X-ray Absorption Fine Structure
XANES: X-ray Absorption Near-edge Structure
TEM: transmission electron microscopy

Typical X-ray Absorption Spectrum

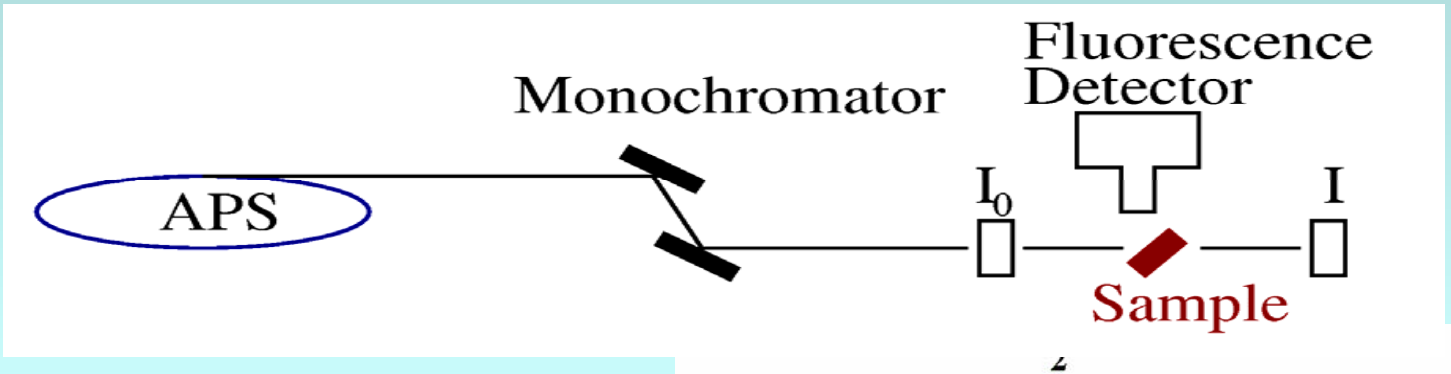
- At the **ionization energy Eo** of the core electron, there is a sudden increase in X-ray absorbance

XAS absorption spectrum is divided into two regions:

- At < ~30 eV above Eo is the region called X-ray Absorption Near Edge Structure **XANES**
- At > ~30 eV above Eo there are fine oscillations in the absorbance profile. This is the Extended X-ray Absorption Fine Structure **EXAFS**

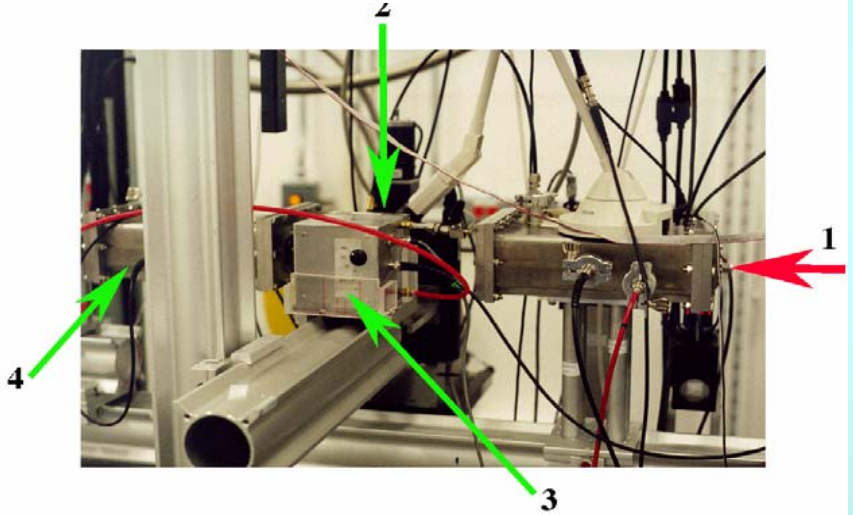


XAFS/XANES Experiment



The experimental setup:

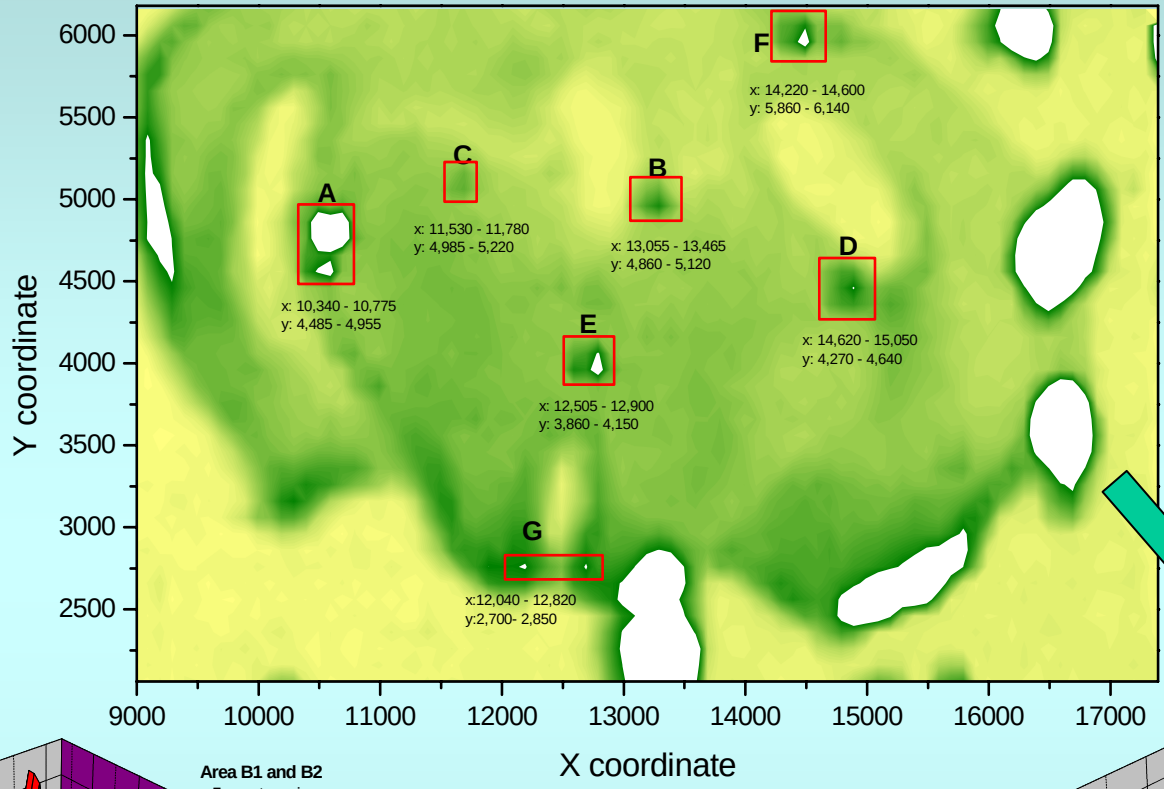
1. **beam direction**
2. **sample position**
3. **fluorescence detector**
4. **transmission detector.**



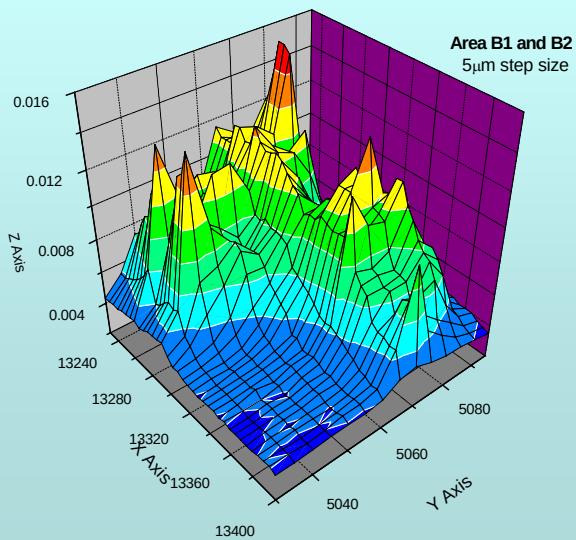
Why Synchrotron X-ray Source?

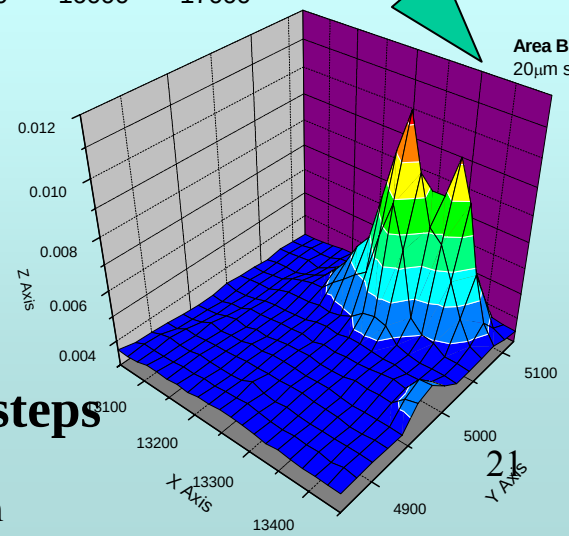
- A high x-ray flux
- A broad spectral range
- High stability in flux, energy beam position

Transgenic R6/2 mouse brain tissue



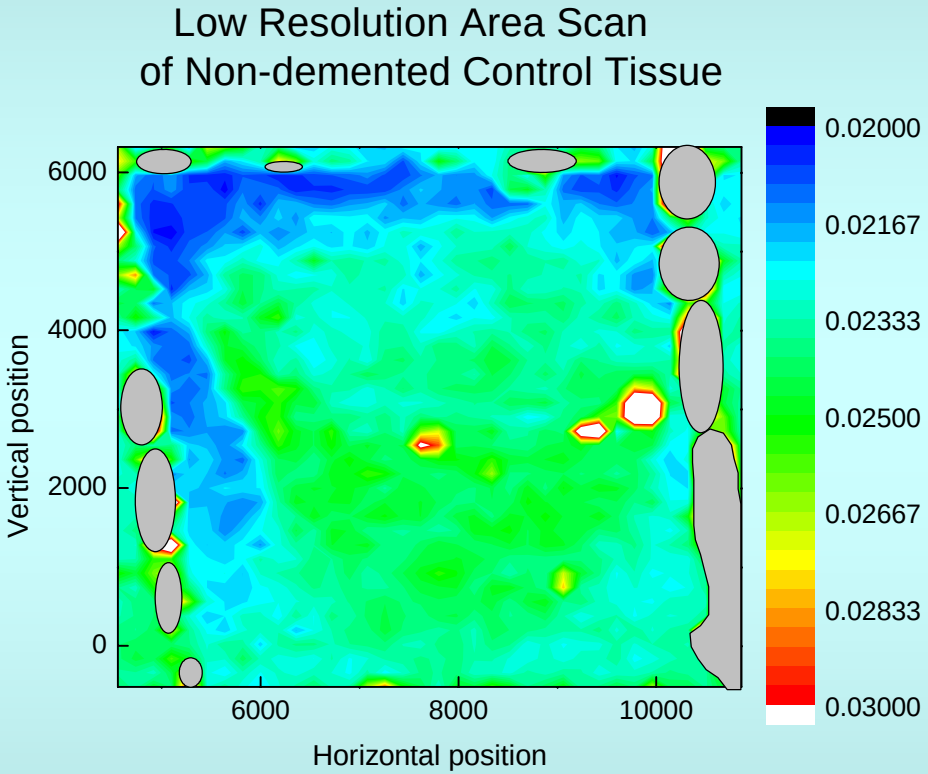
100μm steps

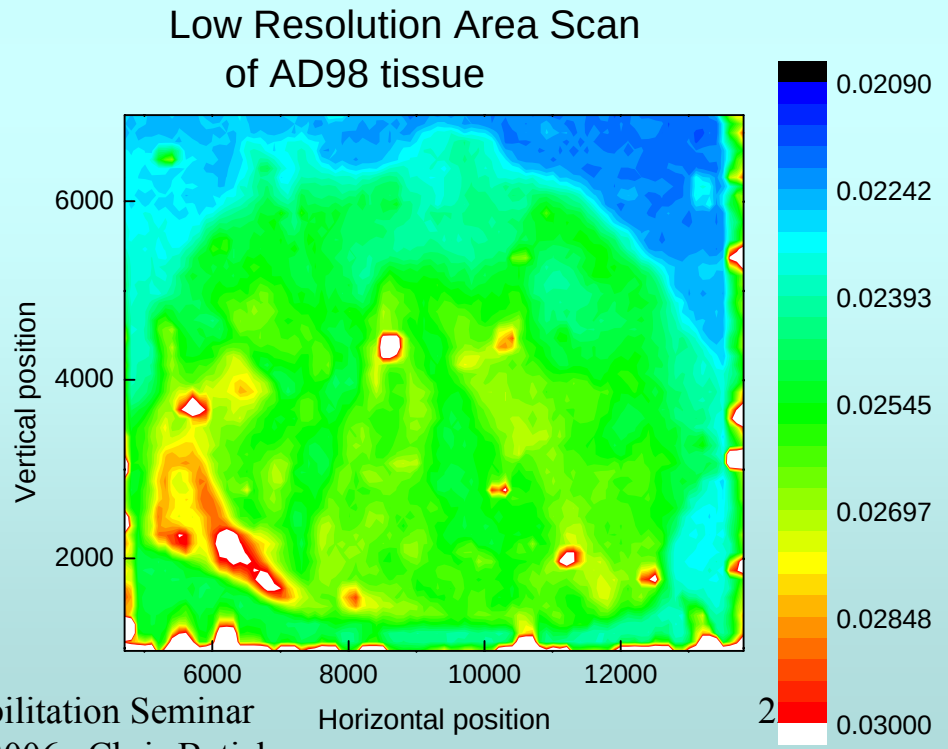




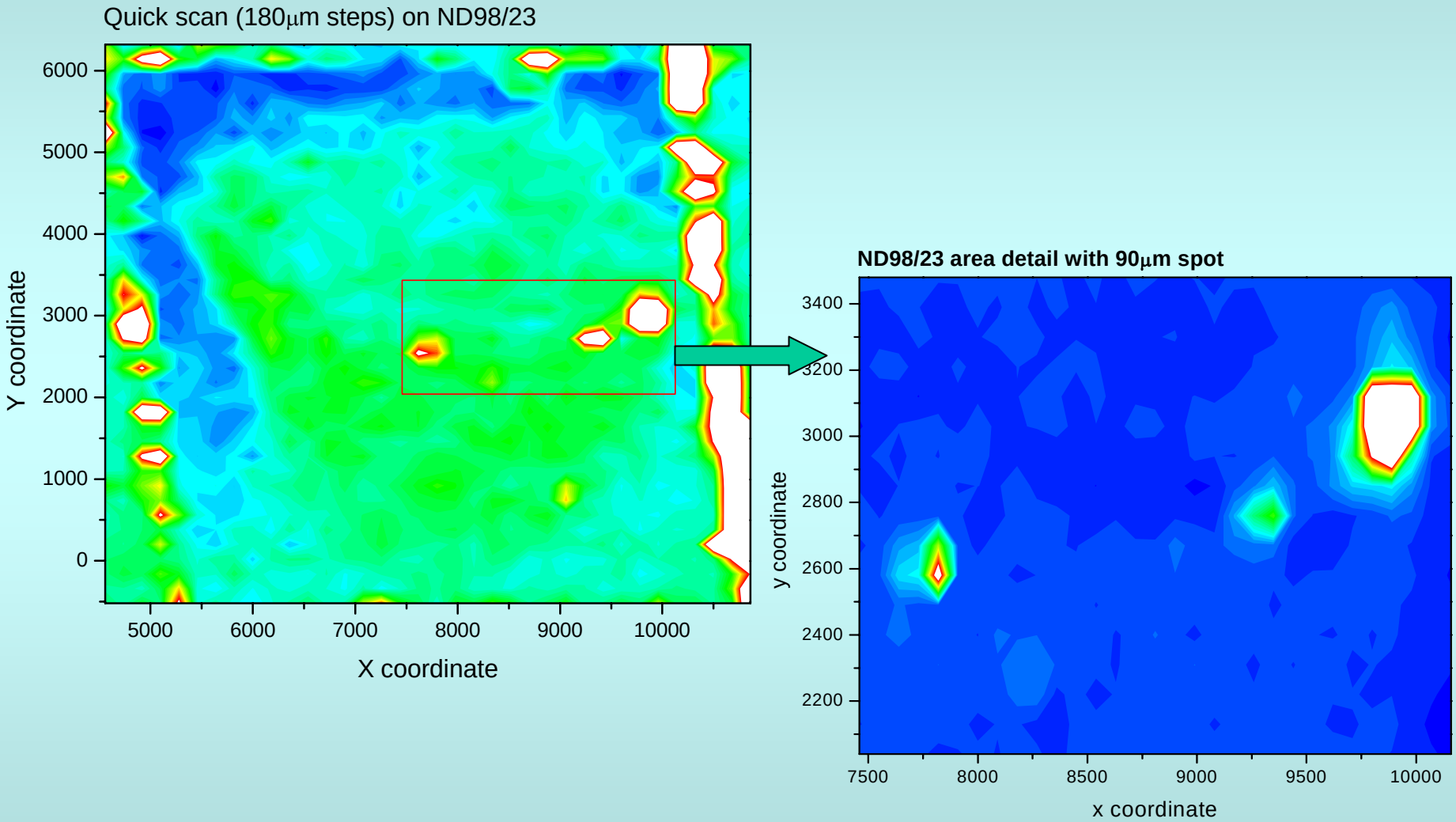
5μm steps **20μm steps**

Comparison of Human Non-demented and Alzheimer's Tissue

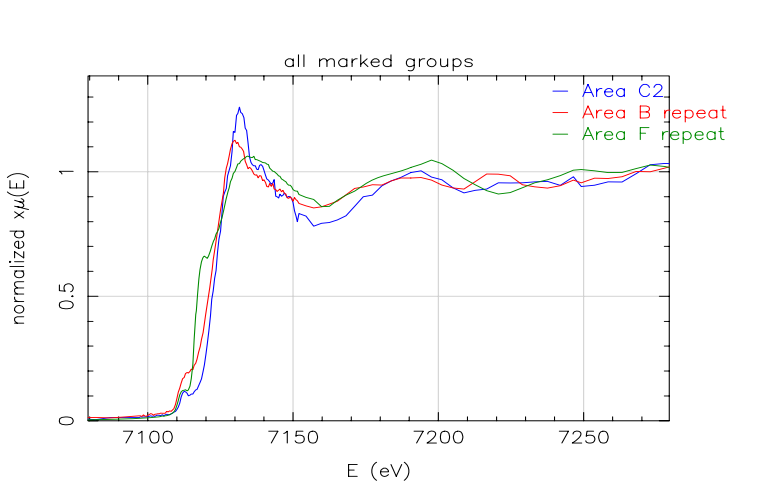


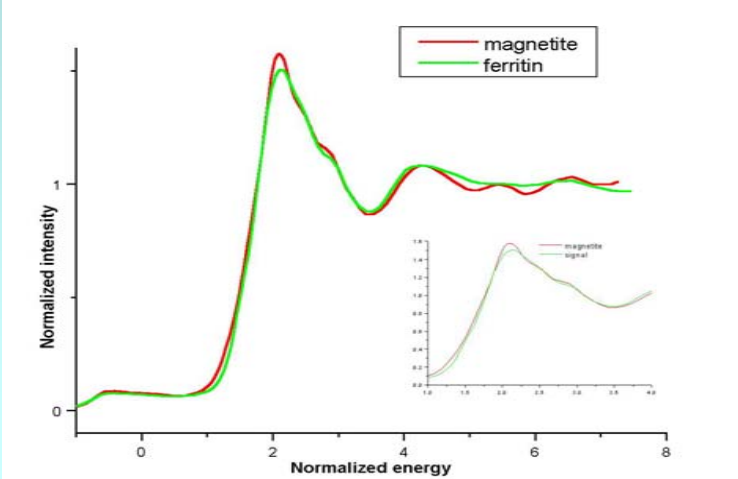


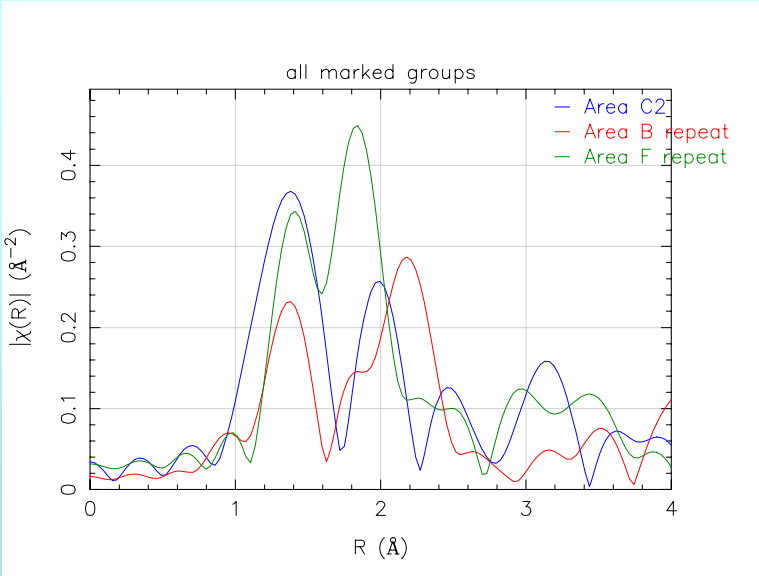
Human non-demented brain tissue

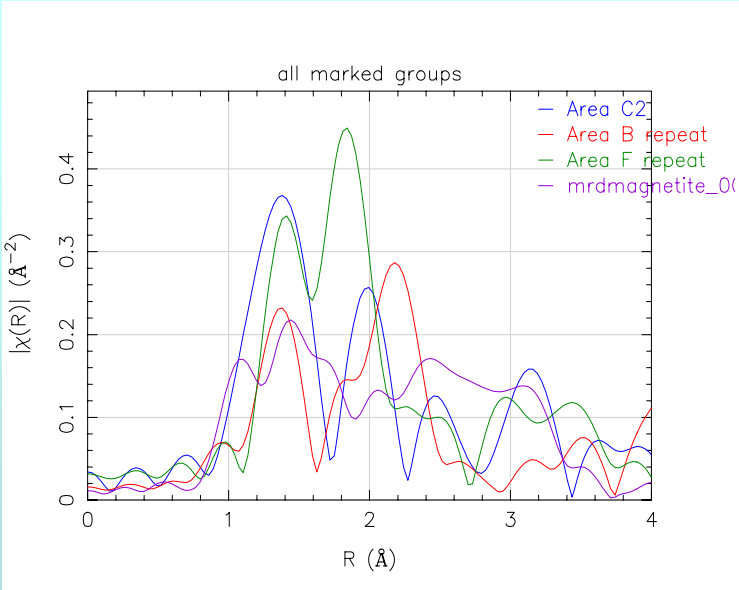


XAFS Collected Spectra Comparison with Magnetite

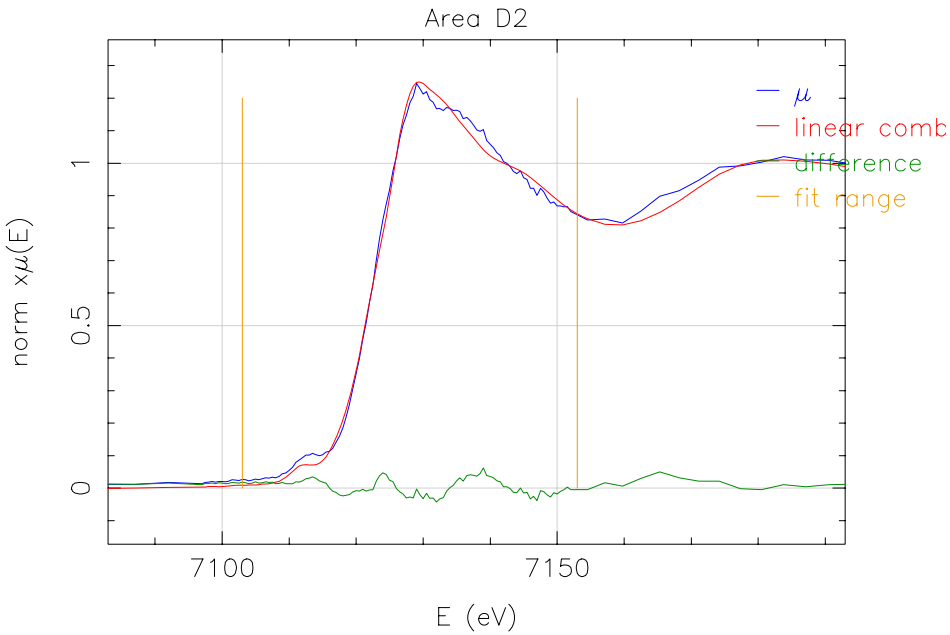


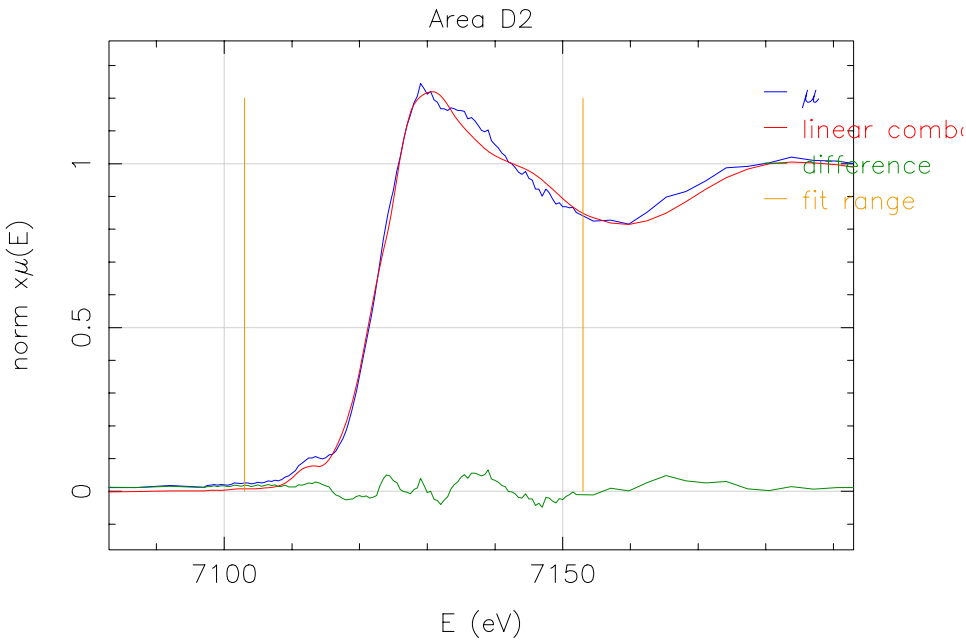




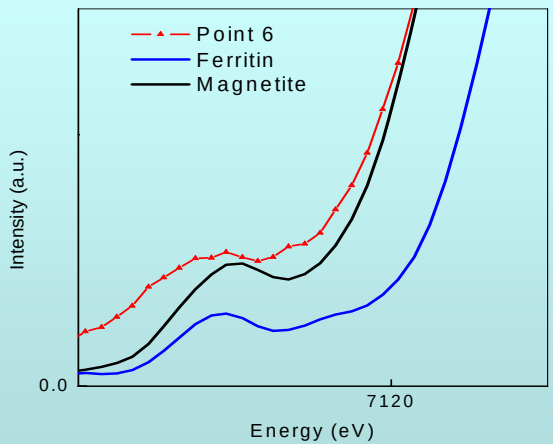


Use of Standards for fitting R6/2 XANES spectra





Ferritin 0.386
Magnetite 0.430
Hematite 0.184

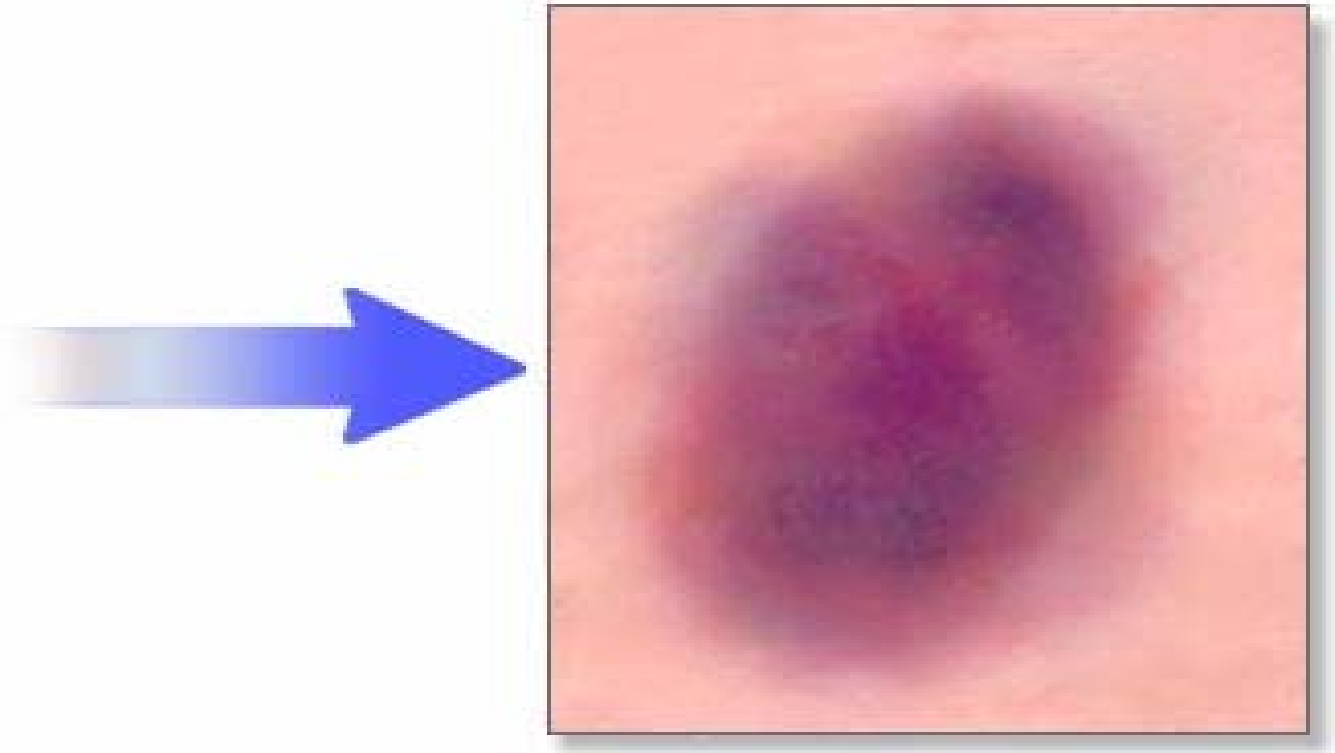


Ferritin 0.376
Magnetite 0.432
Maghemite 0.192

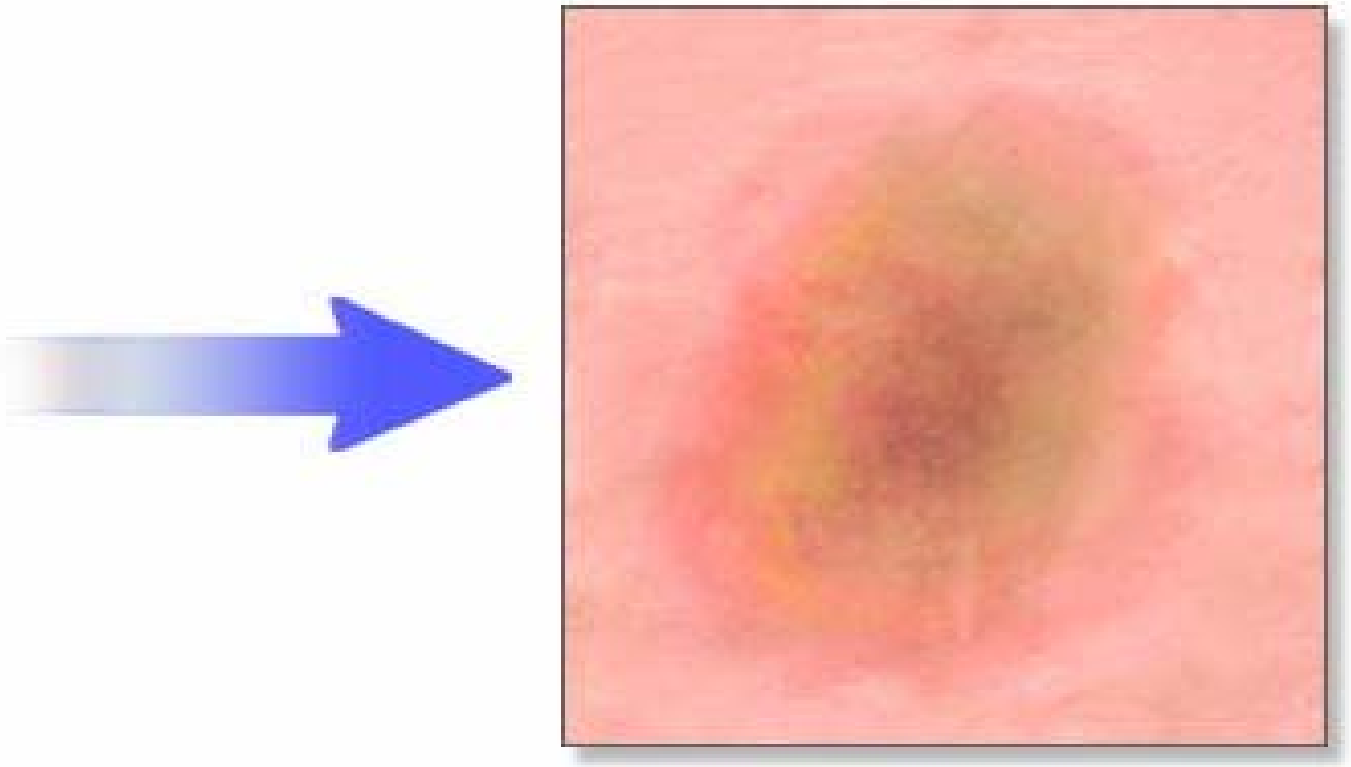
Iron containing brain specimens to develop techniques and understand the types of deposits:

- **Pigeons (done)**
- **Transgenic mouse models of disease:**
  R6/2 mouse (mostly done)
  GFAP-IL6 mouse (inflammatory model, in progress)
- **Human AD brain tissue from autopsy (in progress)**

Where do these deposits come from?

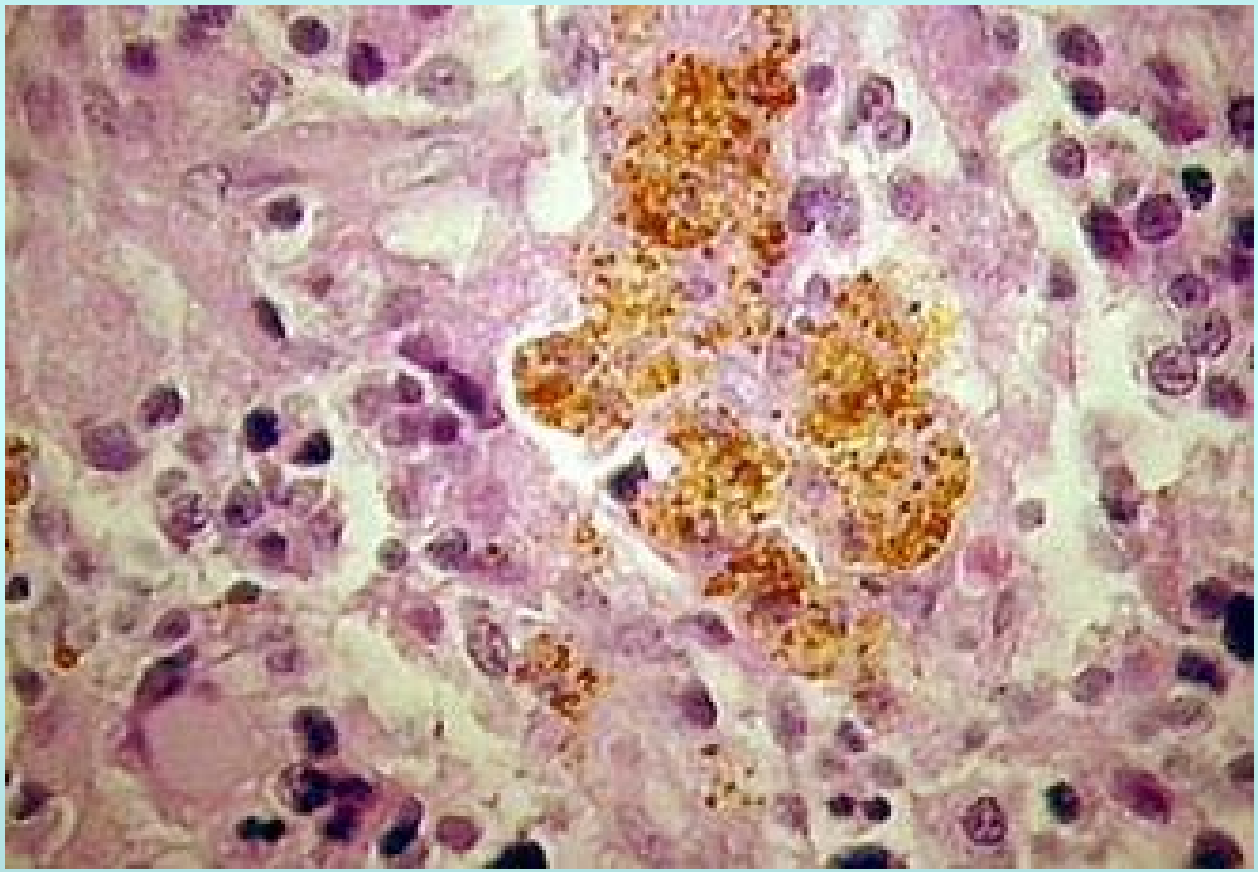


http://health.allrefer.com/pictures-images/bruise-healing-series.html



Then a greenish-yellow color, until it eventually fades away

Hemosiderin in glioblastoma
(http://medlib.med.utah.edu/kw/sol/sss/subj2.html)



Hemosiderin is another iron-storage complex. Its molecular nature remains poorly defined, but it is always found within cells (as opposed to circulating in blood) and appears to be a complex of ferritin, denatured ferritin and other material. The iron within deposits of hemosiderin is, at best, very poorly available to supply iron when needed. Hemosiderin is most commonly found in macrophages and is especially abundant in situations following hemorrhage, suggesting that its formation may be related to phagocytosis of red blood cells and hemoglobin.

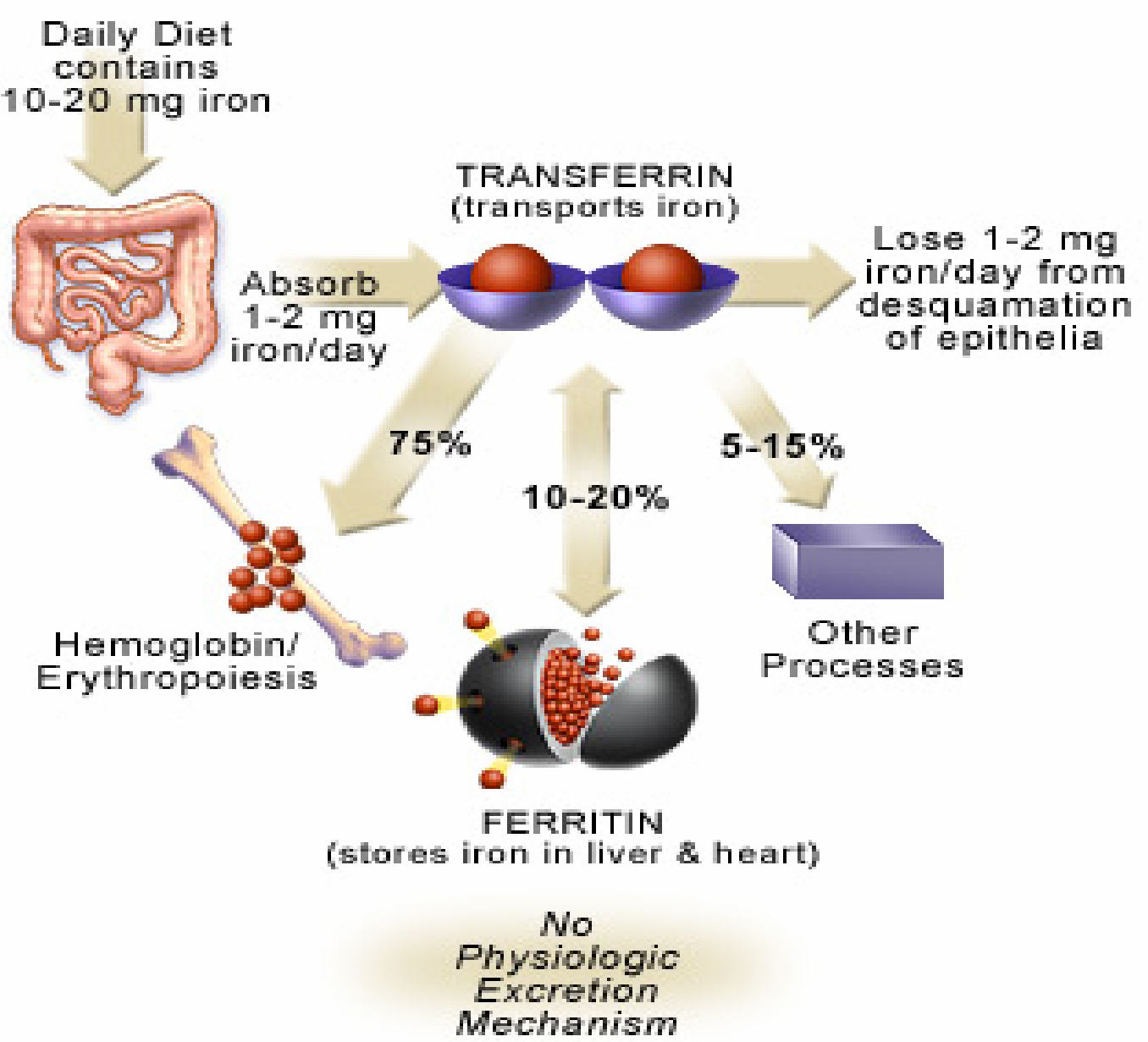
http://arbl.cvmbs.colostate.edu/hbooks/molecules/ferritin.html

Neuroradiology. 2000 Feb;42(2):81-4.

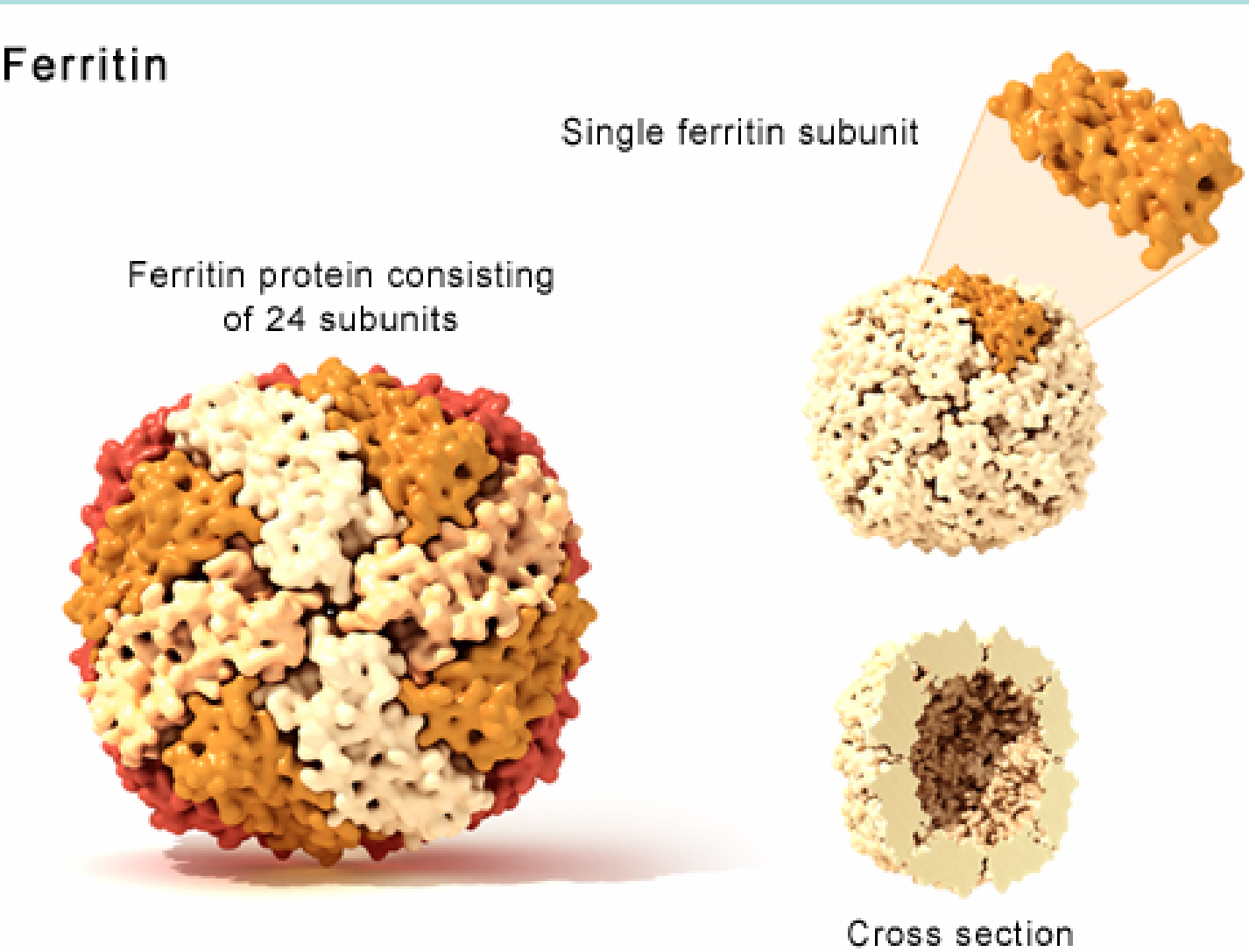
How often is haemosiderin not visible on routine MRI following traumatic intracerebral haemorrhage?

Wardlaw JM, Statham PF.

- "We performed routine MRI (spin-echo T2- and proton-density weighted images) in 116 survivors of moderate to severe head injury, 1-5 years after injury. We reviewed the images blindly and correlated them with CT in the acute stage, to determine how many haemorrhages from the acute stage were identifiable by virtue of haemosiderin deposition on late MRI. Of 106 haemorrhages in 78 patients on CT at the time of injury, 96 **(90 %) were visible as haemosiderin on late MRI."**



http://www.cdc.gov/hemochromatosis/training/pathophysiology/iron_cycle_popup.htm


Ferritin
Single ferritin subunit
Ferritin protein consisting of 24 subunits
Cross section
U.S. National Library of Medicine

- **Natural antioxidants may prevent posttraumatic epilepsy: a proposal based on experimental animal studies.**

  Mori A, Yokoi I, Noda Y, Willmore LJ.

  Acta Med Okayama. 2004 Jun;58(3):111-8.

What kind of damage does OH radical cause?

- Only reacts with what is “near” the source.
- DNA cleavage
- Cell membrane disruption
- Protein cleavage and denaturization

- i.e., cell death,
- leaving behind [encased?] iron in a cross-linked protein-containing matrix

What to do?

- Catalysts can be poisoned (inactivated)
- Iron can be removed (chelated?)
- ??

At UF

Albina Mikhaylova, PhD

Henry Aldrich, PhD

Mark Davidson, PhD

Thomas Eskin, MD

Wolfgang J. Streit, PhD

EM Core Facility

At Keele University, UK

Jon Dobson, Keele, PhD

Joanna Collingwood, PhD

funding from NIH
and MBI seed grant